WORLD INTELLECTUAL PROPERTY ORGANIZATION
INTERNATIONAL APPLICATION PUBLISHED PURSUANT TO THE
PATENT COOPERATION TREATY (PCT)

(51) International Classification of the Patents: C12Q 1/68 A1

(11) International Publication No. WO 96/33287

(43) International Publication Date: October 24, 1996 (10/24/96)

(21) International Application No.: PCT/FR96/00592

(22) International Filing Date: April 18, 1996

(81) Designated Countries: Canada, Japan, US, European Patent (Austria, Belgium, Switzerland, Germany, Denmark, Spain, Finland, France, United Kingdom, Ireland, Italy, Luxembourg, Monaco, Netherlands, Portugal, Sweden)

(30) Priority-related Information:
96/04590 April 18, 1995 France

Published:
With international search report.
Before scheduled deadline for amendment of claims; will be republished if such amendments are received.

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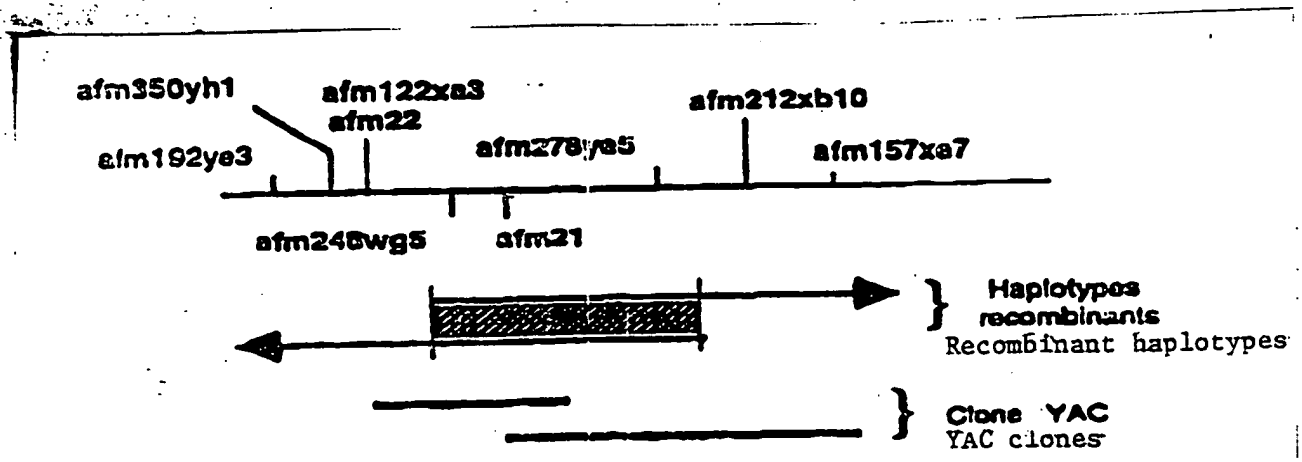
(54) Title: JUVENILE GLAUCOMA DETECTION PROCESS



(57) Abstract: The invention concerns a process for detecting a predisposition to juvenile glaucoma in a person, the process being characterized in that microsatellite markers associated with the occurrence of juvenile glaucoma in the person's family are characterized in a biological sample taken from said person. These markers are afm350yhl, afm122xa3, ngal, afm21, afm248wg5, afm278ye5, afm212xb10, afm157xe7, and NGA5 of chromosome 1q21q31.

JUVENILE GLAUCOMA DETECTION PROCESS

The present invention concerns, in particular, case finding of a family predisposition to juvenile glaucoma, as well as possible revelation of the gene responsible for this disorder.

Juvenile glaucoma affects nearly 100,000 persons in France and is thus one of the main causes of blindness in that country.

As indicated by the name, the initial symptoms often appear before the age of 40 and are manifested by a reduced field of vision. This painless, very gradually-progressing condition is frequently not grasped by the patient, and is detected only at an advanced, often irreversible stage, at which it will progress toward blindness.

Juvenile glaucoma is caused by a combination of ocular hypertension and damage to the optic nerve. At present, efficacious treatments, especially medical and surgical in nature, do exist; they make it possible to halt the disease or slow the progress thereof. However, such treatments prove highly effective only when the glaucoma is detected at an early stage. Late detection entails extensive surgeries which may leave handicaps.

For a number of years, the predominant role of genetic factors in the onset of juvenile glaucoma has been revealed. It is now beyond dispute that, in most cases, the disease is transmitted in accordance with the simple dominant Mendelian mode, since the gene in question is located on the long segment of chromosome 1.

That is to say, juvenile glaucoma is linked to the existence on chromosome 1 of a specific gene or structure, the presence of a single copy of which is enough to trigger the disease.

Accordingly, to ensure early detection of this condition, it must be determined whether this gene is present in the patient. Currently, the region in which the gene is located, or the so-called "locus of susceptibility," but not the exact position or structure thereof, is known. A "locus of susceptibility" may contain approximately 10 million bases; accordingly, localization is obviously quite complex, especially since nothing is known about the structure of the gene. As a result, the presence of the gene in question cannot be detected directly, and, for this reason, the diagnosis must involve use of the "microsatellite" technique.

Chromosomes contain sequences that are highly polymorphic; in other words, these sequences are composed differently depending on the individual, even though they lie at the same location on the chromosome; these areas are termed "microsatellites" and have prompted publication of numerous works.

When the disorder is dominant and familial in origin, it is possible to study the correlation between the onset of juvenile glaucoma and an allele of a determinate microsatellite marker. When the correlation rate is high, the presence of the allele of the microsatellite may be held to signify the presence of the disease-producing gene.

Because these microsatellites are positioned in the same area, one can plausibly contemplate revealing the presence of a specific microsatellite, which would be linked to the gene being sought.

Accordingly, it is essential to analyze the patient's family and to have this patient's history available; in other words, for each individual belonging to the family being studied, one must confirm the existence of both the disease and a specific marker, that is, the allele of a

microsatellite, which would necessarily, or very probably, be associated with the disorder.

If the family is sufficiently numerous, it will be possible to determine the markers that are statistically the most frequently present when the disease is detected.

The maximum correlation rate is given by a microsatellite marker located very close to the gene in question. In fact, in this case these two portions of the chromosome are transmitted in their original form to the descendants. On the other hand, if the marker is more distant, it may be separated from the gene at the time of transmission ("crossing-over"), and the presence thereof is not necessarily linked to the presence of the defective gene.

It must, therefore, be understood that, in principle, detection of a microsatellite marker in any given individual is not significant. In fact, lacking any specific heredity, this marker is often of no interest, and it must also be understood that, even when the family history reveals hereditary juvenile glaucoma, the presence of the familial marker from another family is not necessarily a determining factor in predicting the appearance of the disease. Correlation studies must be repeated, even if, when the families are in very close proximity (same region) and even in the absence of known common ancestors, the marker can, in some cases, be a distinguishing factor (lack of linkage correlation between the marker and the disorder).

For this reason, the invention concerns a process for detection of a predisposition to juvenile glaucoma in a patient, which is characterized by the fact that the presence of a microsatellite marker linked to the onset of juvenile glaucoma in the patient's family is detected in a biological sample drawn from the patient, the microsatellite marker being chosen from the following markers: afm350yhl, afm122xa3, ngal, afm21, afm248wg5, afm278ye5, afm212xb10,

afm157xe7, and NGA5 of chromosome 1q21q31.

More specifically, the detected microsatellite marker is preferably located in the locus corresponding to the area located between the afm248wg5 and afm212xb10 markers.

The techniques employed to detected the presence of markers are conventionally known. Detection may be carried out directly, using a probe complementary with the marker; however, use will preferably be made of techniques entailing enlargement of the marker sequence, for example using PCR (Polymerase Chain Reaction) techniques which use initiators, one of which is marked with a fluorochrome.

To simplify detection of the various markers in question, use may be made of the "multiplex" PCR technique. The underlying principle involves several PCR reactions occurring simultaneously and based on a single sample of genomic DNA. The intent is to save reagents and the DNA awaiting typing, and to decrease the risk of error by reducing the number of handling operations.

To this end, combinations of pairs of initiators are tested in pairs. Then, if the results permit, a third pair is added, and so on.

As previously indicated, the presence of this microsatellite marker signals only a predisposition to the occurrence of juvenile glaucoma; such a finding is valid when, after analysis of the family history, a link has been established between the presence of these markers and the onset of juvenile glaucoma.

The invention also relates to the DNA sequence located between the locus containing the markers afm122xa3 and afm212xb10, preferably afm122xa3 and NGA5, this portion being

linked to the juvenile glaucoma system.

Indeed, by virtue of previous advances, the gene implicated in juvenile glaucoma can be located with precision.

The use of the microsatellite markers described above to characterize recombinant haplotypes within the large families makes it possible to arrange the markers in relation to each other. In subjects afflicted with glaucoma, these recombinant haplotypes allow enhanced delineation of the chromosomal region containing the disease-producing gene. Accordingly, a region measuring three centimorgans has been demarcated (Figure 1). Figure 2 shows a more detailed map of the region D1S210 - L854B9, in which the gene predisposing to juvenile glaucoma is found.

The markers described above have allowed identification of artificial yeast chromosomal clones covering the region. Accordingly, 25 YACs extending over a maximum distance of 3 Mbases delineate a first-level pathway.

The gene may then be isolated by:

- the simultaneous search for new polymorphic markers and new recombinant haplotypes, which will make it possible to narrow even further the region containing the gene,
- the direct search for genes using several techniques:
  * transcript selection by means of hybridization
  * exon trapping
  * phylogenetically-preserved sequences
  * CpG islets

* triplet expansion.

Finally, the genotyping process described will allow identification of the families in which the glaucoma gene located on chromosome 1 is not involved, thus making it possible to locate other glaucoma-predisposing genes in other areas of the genome.

Other features and techniques for implementation of the process will emerge from a reading of the following example.

Example

Technique for Typing Human Genomic DNA Using 9 Microsatellite Markers of the Region 1q21q31

A) DNA was extracted from peripheral venous blood after cell lysis, protein digestion, organic division, and, finally, alcohol precipitation.

The blood (20 ml) was taken by peripheral venous puncture in a tube containing EDTA.

The blood was diluted using a volume of bidistilled water. After 10 minutes, the cells were collected by centrifugation at 1,600 g for 10 minutes. This procedure was repeated.

The white cells were lyzed in the presence of 20 ml CLB buffer (10 mM Tris, pH 7.6, 5 mM $MgCl_2$, 0.32 M sucrose, 1% Triton X-100 (v/v). The nuclei were collected by centrifugation at 1,600 g for 10 minutes. This procedure was repeated.

The nuclei were washed once in RSB buffer (10 mM Tris, pH 8, 10 mM NaCl, 10mM EDTA).

The residue was put back in suspension in 2 ml RSB buffer, to which 1% sodium lauryl sulfate and proteinase K (200 µg/ml) were added. The mixture was incubated at 55° C for at least 3 hours and periodically stirred.

The DNA solution thus obtained was then extracted using a volume of phenol balanced with a 50 mM Tris buffer, pH 8. This procedure was repeated and supplemented by extraction using a quantity of chloroform/isoamyl alcohol (24:1 v/v).

The DNA was precipitated out using a quantity of isopropanol, washed in 70% ethanol, dried, and, finally, put back in suspension in 1 ml TE buffer (10 mM Tris, pH 8, and 0.5 mM EDTA).

The DNA concentration was evaluated by measuring absorption at 260 mm, using 50 µg/ml DNA per unit of absorption. The DNA concentration was then adjusted to 200 µg/ml.

B) Expansion of Genomic DNA for Fluorescent Microsatellite Markers

Nine microsatellites were used to determine the haplotypes associated with the region 1q21q31 on the long segment of chromosome 1. The list of these microsatellites, with a description of the initiators (sequence and addition of a fluorochrome) is given in Table 1.

The expansion conditions common to these markers were as follows:

Reaction mixture:

- Genomic DNA (200 µg/ml)	1 µl
- Triphosphate nucleotides (1.25 mM each)	4 µl
- Initiators (10 picomoles/µl each)	2 x 1 µl
- Taq DNA Polymerase (R) (5 U/µl)	0.05 µl
- PCR buffer concentrated 10 times	2.5 µl
- H2O quantity sufficient to make:	25 µl

Composition of the 10 x PCR buffer: 0.1 M Tris, pH 8.3 at 20° C, 0.5M KCL, 15 mM $MgCl_2$, 1 mg/ml gelatin (Sigma G2500 (R)).

To expand the nga1 marker, the TaqStart antibody (R) (Clontech) was added to the final 0.056 μM concentration (giving an antibody/enzyme mole ratio of 28:1).

Expansion was carried out in a Techne (R) PHC-3 thermocycling unit with heating cover. After heating to 94° C for five minutes, 30 cycles were carried out. Each cycle consisted of two segments lasting one minute each, at 55° C and 94° successively. A final lengthening segment lasting 2 minutes at 72° C ended the expansion process.

Table. List of Microsatellite Markers Used to Haplotype the Region of the Juvenile Glaucoma Gene on Chromosome 1q21q31.

MARKER (size in pb)	INITIATOR NUCLEOTIDE SEQUENCE (upper, then lower)
afm350yh1 (189 bp)	JOE-5'-TCTTCCCACCACTGCC 5'-TGTATTCCTACTGCCCA
afm122xa3 (D1S210) (121 pb)	FITC-5'-CCTCAGTTCATTCCCATAA 5'-AGCTGAATCTCACCCAATAACTA
nga1 (200 bp)	5'-JOE CCAACTGAGAATTCTATATTTAACC 5'-TCTGGTAGGGCAGATCTGCTAGAA
afm21 (121 bpN)	JOE-5'-CCTTCCTTTCTAAGGCTG 5'-TCTTATCAGTCAGGCA
afm248wg5 (D1S452) (223 pb)	FITC-5'-TAATGGGTTCAGTGGACCTT 5'-TGCAGTTCCATATTCCAGGT
afm278ye5 (240 bp)	JOE-5'-TGAGCCGAGATTGAGCC 5'-CCAGGTCAGAGATGTTGG
afm212xb10 (D1D242) (219 bp)	5'-TAMRA-TCTACCACTTGAATTCCTGT 5'-ACCACTCCAGTTTGAGCAAC
afm157xe7 (D1S218) (274 pb) NGA5 (L854B9)	5'-FAM- TGTAAAAGCAAACTGTAGACGAT 5'-TTTATGTTATCACCAAGGCTTCT CTGAAACTGAGATAGGAGTCC GAAATGGGAGTTGAGTTACCC

The expansion products (2μl) from a single individual were collected, coprecipitated in ethanol (2.5 volumes, 2 hours at -20°C), and allowed to begin migrating along a single acrylamide gel (6 96) - urea (8M) pathway, after being placed back in suspension in a filler buffer (2 μl formamide, 0.5 μl ABI Blue (R), 0.5 μl GeneScan 2500 Rox, 1 μl H2O) and after thermal denaturation. Electrophoresis was performed in an ABI 373 automatic sequencer under 30 Watts power for eight hours, the laser beam being positioned at a height of 24 cm.

The raw data were analyzed using the GeneScan Analysis (ABI) software. The fluoresence peaks were identified using the Genotyper software (ABI), thus making it possible to assign the alleles of the microsatellite markers.

Interpretation of Findings

These typing results were interpreted as a function of context:

- in a family in which a haplotype predisposing to glaucoma associated with this region of chromosome 1 had already been characterized, comparison of the alleles from the sample tested with those from the family haplotype allowed simple determination of the presence or absence of the predisposing gene. Given the current state of knowledge, the presence of this gene entails an estimated 80% risk of developing the disease. When the gene is lacking, the risk is the same as that occurring in the general population, or 1-2 % according to epidemiological studies.
- in a family in which a haplotype predisposing to the glaucoma gene and associated with this region of chromosome 1 has not already been characterized, the probability of the presence of the

disease-producing gene was estimated using LINKAGE software.

The findings collected from different families demonstrated the excellent predictive capability of the markers used.

LIST OF SEQUENCES

(1) GENERAL INFORMATION:

(I) APPLICANT

A) NAME: INSTITUT NATIONAL DE LA RECHERCHE MEDICALE (INSERM)
B) STREET: 101 RUE DE TOLBIAC
C) CITY: PARIS
D) COUNTRY: FRANCE
E) ZIP CODE: 75013

(ii) TITLE OF THE INVENTION: JUVENILE GLAUCOMA DETECTION PROCESS
(iii) NUMBER OF SEQUENCES: 18
(iv) FORM DECIPHERABLE BY COMPUTER:
A) MEDIUM: Floppy disk
B) COMPUTER: MACINTOSH APPLE
C) OPERATING SYSTEM: MAC-OS SYSTEM 7
D) SOFTWARE: WORDPERFECT 2.0

(2) DATA FOR SEQUENCE ID NO. 1:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 16 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR" afm350yh1

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 1
TCTTCCCACC ACTGCC

(2) DATA FOR SEQUENCE ID NO. 2:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 17 pairs of bases
B) TYPE: nucleotide

C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR"
afm350yh1

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 2
TGTATTCCTA CTGCCCA

(2) DATA FOR SEQUENCE ID NO. 3:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 19 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR"
afm122xa3

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 3
CCTCAGTTCA TTCCCATAA

(2) DATA FOR SEQUENCE ID NO. 4:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 23 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR"
afm122xa3

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO.
AGCTGAATCT CACCCAATAA CTA

(2) DATA FOR SEQUENCE ID NO. 5:

(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 25 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR" nga1

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 5
CCAACTGAGA ATTCTATATT TAACC

(2) DATA FOR SEQUENCE ID NO. 6:

(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 24 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR" nga1

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 6
TCTGGTAGGG CAGATCTGCT AGAA

(2) DATA FOR SEQUENCE ID NO. 7:

(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 18 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR" afm21

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 7
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(2) DATA FOR SEQUENCE ID NO. 8:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 16 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR" afm21

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 8
TCTTATCAGT CAGGCA

(2) DATA FOR SEQUENCE ID NO. 9:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 20 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR" afm248wg5

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 9:
TAATGGGTTC AGTGGACCTT

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(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 20 pairs of bases
B) TYPE: nucleotide
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D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid

A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR"
afm248wg5

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(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 17 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR"
afm278ye5

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 11:
TGAGCCGAGA TTGAGCC

(2) DATA FOR SEQUENCE ID NO. 12:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 18 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR"
afm278ye5

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 12:
CCAGGTCAGA GATGTTGG

(2) DATA FOR SEQUENCE ID NO. 13:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 20 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single

D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR" afm212xb10

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 13:
TCTACCACTT GAATTCCTGT

(2) DATA FOR SEQUENCE ID NO. 14:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 20 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR" afm212xb10

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 14:
ACCACTCCAG TTTGAGCAAC

(2) DATA FOR SEQUENCE ID NO. 15:
(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 23 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR" afm157xe7

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 15:
TGTAAAAGCA AACTGTAGAC GAT

(2) DATA FOR SEQUENCE ID NO. 16:

(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 23 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR"
afm157xe7

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 16:
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(2) DATA FOR SEQUENCE ID NO. 17:

(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 21 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "UPPER NUCLEOTIDE INITIATOR"
NGA5

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 17:
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(2) DATA FOR SEQUENCE ID NO. 18:

(I) SEQUENCE CHARACTERISTICS:
A) LENGTH: 21 pairs of bases
B) TYPE: nucleotide
C) NUMBER OF STRANDS: single
D) CONFIGURATION: linear

(ii) TYPE OF MOLECULE: Other nucleic acid
A) DESCRIPTION: /desc = "LOWER NUCLEOTIDE INITIATOR"
NGA5

(xi) DESCRIPTION OF THE SEQUENCE: SEQUENCE ID NO. 18:
GAAATGGGAG TTGAGTTACC C

CLAIMS

1. Process for detection of a predisposition to juvenile glaucoma in an individual, wherein, in a biological sample drawn from said individual, microsatellite markers linked to the onset of juvenile glaucoma in the individual's family are characterized, the microsatellite markers being afm350yhl, afm122xa3, nga1, afm21, afm248wg5, afm278ye5, afm212xb10, afm157xe7, and NGA5 on the chromosome 1q21q31.

2. Process according to claim 1, wherein the marker is located on the locus corresponding to the region located between markers afm248wg5 and afm212xb10.

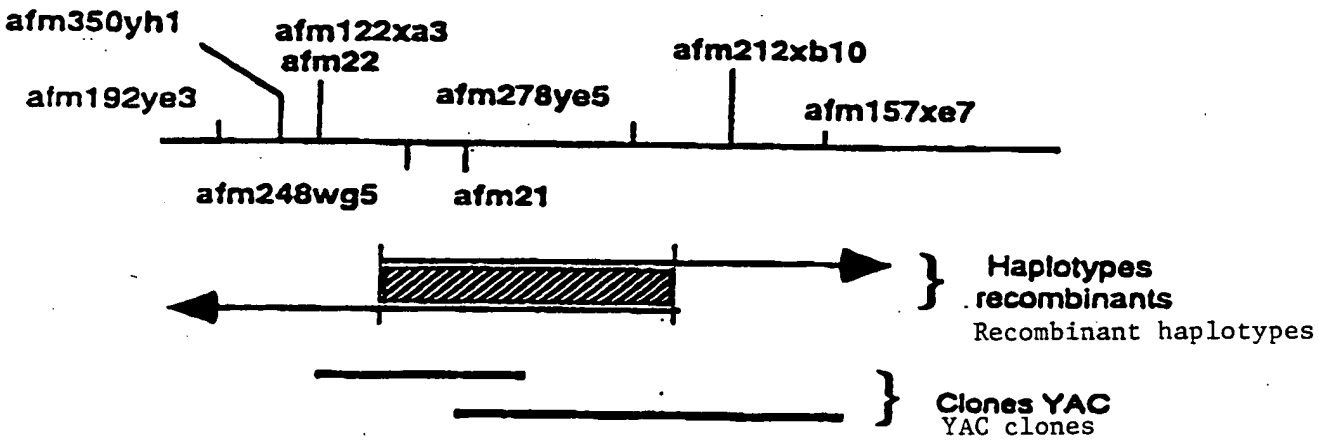
3. Process according to either of claims 1 and 2, wherein the presence of said marker is detected by expansion of the marker area.

4. Process according to claim 3, wherein expansion is effected by means of PCR.

5. Process according to claim 4, wherein use is made of multiplex PCR expansion.

6. DNA sequence located between the locus corresponding to markers afm122xa3 and afm212xb10 and linked to the onset of juvenile glaucoma.

7. DNA sequence according to claim 6, wherein said sequence is located between markers afm122xa3 and NGA5.


afm350yh1
afm122xa3
afm22
afm212xb10
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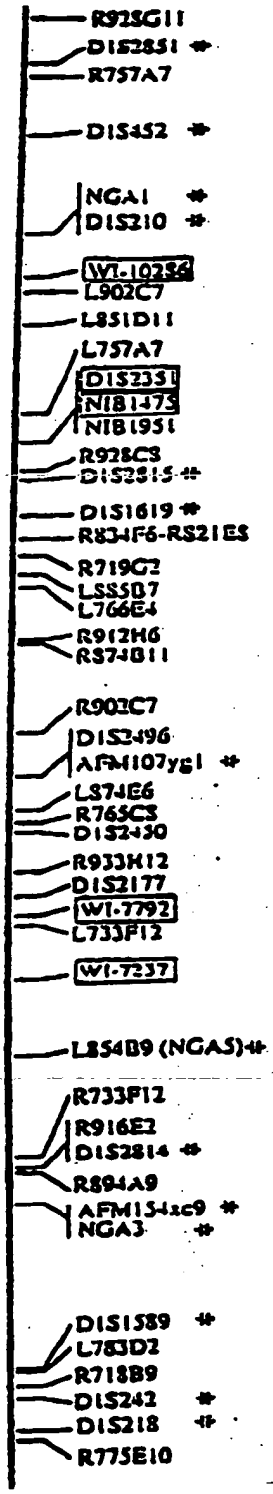
afm21
Haplotypes recombinants
Recombinant haplotypes
Clones YAC
YAC clones

FIGURE 1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R928C3
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DIS1619
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R719C2
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L766E4
R912H6
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R894A9
AFM154xc9
NGA3
DIS1589
L783D2
R718B9
DIS242
DIS218
R775E10

FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FR 96/00592

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	44TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS, MONTREAL, QUEBEC, CANADA, OCTOBER 18-22, 1994. AMERICAN JOURNAL OF HUMAN GENETICS 55 (3 SUPPL.). 1994. A203. ISSN: 0002-9297. XP002010897 SEGHATOLESLAMI M R ET AL: "Fine mapping of juvenile primary open angle glaucoma (POAG) on 1q21 -q31 and exclusion of adult-POAG from the respective region." see abstract --- -/--	1-6

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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Date of the actual completion of the international search	Date of mailing of the international search report
14 August 1996	26.08.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Molina Galan, E

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	44TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS, MONTREAL, QUEBEC, CANADA, OCTOBER 18-22, 1994. AMERICAN JOURNAL OF HUMAN GENETICS 55 (3 SUPPL.). 1994. A201. ISSN: 0002-9297. XP002010898 RAYMOND V ET AL: "A single gene for juvenile and middle-age onset open-angle glaucomas confined within a small interval on chromosome 1q." see abstract	1-6
Y	C R ACAD SCI III, (1994 JUN) 317 (6) 565-70, XP000564959 MEYER ET AL.: "Liaison du glaucome juvenile au chromosome 1q dans deux familles francaises." see the whole document	1-6
Y	GENOMICS, (1994 MAY 15) 21 (2) 299-303., XP002010899 WIGGS ET AL.: "Genetic linkage of autosomal dominant juvenile glaucoma to 1q21-q31 in three affected pedigrees." see the whole document	1-7
Y	NATURE GENET. 7:246-339(1994), XP000560918 GYAPAY ET AL.: "The 1993-1994 Genethon human genetic linkage map" see the whole document	1-7
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A	44TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS, MONTREAL, QUEBEC, CANADA, OCTOBER 18-22, 1994. AMERICAN JOURNAL OF HUMAN GENETICS 55 (3 SUPPL.). 1994. A206. ISSN: 0002-9297, XP002010903 WIGGS J ET AL: "Further Evidence for a Locus for Autosomal Dominant Juvenile Glaucoma on Chromosome 1q and Evidence for Genetic Heterogeneity." ---	
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